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Original paper

Diagnosis of spontaneous bacterial peritonitis in children using leukocyte esterase reagent strips and granulocyte elastase immunoassay

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Abstract

Aim of the study: We aimed to assess the utility and rapidity of granulocyte elastase (GE) latex immunoassay and leukocyte esterase (LE) reagent strips for the diagnosis of spontaneous bacterial peritonitis (SBP) in hepatic children with ascites.

Material and methods: This study included 80 ascitic fluid (AF) samples from 45 patients with chronic liver diseases. They were divided into 2 groups (SBP and non-SBP groups). White blood cells > 500 cell/mm^3 or polymorphonuclear leukocytes > 250 cell/mm^3 were considered as a positive result for the diagnosis of SBP. The AF obtained at bedside was immediately tested with an LE enzyme reagent strip, and another sample was aliquoted for measurement of serum GE.

Results: On doing LE dipstick strips, all the non-SBP group gave no coloration with LE strips while 62% of the SBP group gave coloration. LE strips had accuracy of 86.25% in differentiating SBP and non-SBP at a cut-off value of 1 (color grade 1). The diagnostic performance of GE in differentiating SBP and non-SBP was assessed and showed accuracy of 70% for a cutoff value of 123.5 ng/ml.

Conclusions: Application of LE reagent strips is a bedside, rapid, easy-to-use, and inexpensive method for diagnosis of SBP. It has an accuracy of 86.25% in differentiating SBP and non-SBP, which is higher than more complicated and delayed methods such as GE latex immunoassay, which has an accuracy of 70%.

Key words: spontaneous bacterial peritonitis, granulocyte elastase, leukocyte esterase.

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Introduction

Spontaneous bacterial peritonitis (SBP) arises almost exclusively in patients with cirrhosis. It has been described as an ascitic fluid (AF) infection in the absence of any intra-abdominal, surgically treatable source of infection. It is diagnosed when there is an elevated AF absolute polymorphonuclear leukocyte (PMN) count with or without positive AF culture (i.e. ≥ 250 cells/mm^3) [1].

The count of AF PMNs is operator-dependent and lysis of PMN cells during transport to the laboratory may lead to false-negative results. Furthermore, AF culture is insensitive and leads to delay in diagnosis by several days. A delay in antibiotic therapy entails a high mortality rate; therefore, considerable efforts have been invested in developing a rapid and reliable test for the diagnosis of SBP [2].

Using bedside, rapid, easy-to-use, and inexpensive methods for diagnosis of SBP will help in early diagnosis and appropriate intervention [3].

The aim of this study is to assess the utility of granulocyte elastase (GE) immunoassay and rapidity of leucocyte esterase (LE) reagent strips for the diagnosis of SBP in cirrhotic children with ascites.

Material and methods

This case control study included 80 AF samples from 45 patients with chronic liver diseases. They were divided into 2 groups (SBP and non-SBP groups). The number of paracentesis procedures per patient ranged from one to four, with an average of 2 AF samples/patient. The SBP group included 40 AF samples with PMN count in AF ≥ 250 cells per mm^3 with or without culture positive or positive AF culture with PMN < 250 per mm^3. Non-SBP group: included 40 AF samples with PMN count in AF < 250 cells per mm^3 with AF culture negative.

All patients in this study were subjected to full history taking, thorough clinical examination, and routine investigations. AF samples were carried out under strict sterile conditions with the patient in a supine position, ultrasound guided or during tapping of a large amount of ascites.

AF samples were investigated for AF culture and sensitivity, and total leucocytic count (TLC) with its differential. Biochemical AF tests, such as triglycerides, cholesterol, bilirubin, albumin, total protein, glucose, amylase, lipase and lactic dehydrogenase, were performed.

After the paracentesis, the AF specimen was collected in a clean sterile container. Then the LE test strip (Combur test UX; Roche Diagnostics GmbH, D-68298 Mannheim, Germany) was dipped for no longer than 1 second. After 60 seconds the reaction color in the test area was compared against the color scale (4 grade scale from 0 to 3) on the label.

Human GE was measured by ELISA technique and supplied by BIOSCIENCE, USA, with catalog number BMS269, Affymetrix.

Methods of data analysis and statistical evaluation

Data were collected and entered to the computer using SPSS (Statistical Package for the Social Science) program for statistical analysis version 21 (Armonk, NY: IBM Corp.). Quantitative data will be shown as mean ±SD. Qualitative data will be expressed as frequency and percent. The chi-square test (χ^2) was used to evaluate the association between qualitative variables. The Mann-Whitney test (z) and independent sample t test were used to evaluate the association between 2 sets of quantitative data. The results of comparing the correlation between two continuous variables are indicated by the correlation coefficient (r) using correlation analysis. The clinical performance of GE and LE in differentiating SBP from non-SBP were assessed by the receiver operating characteristic (ROC) curves. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were presented as percentages. The P (probability) value is considered to indicate statistically significance if it is less than 0.05.

Table 1. Etiological diagnosis of the ascitic patients

Etiological diagnosis	Studied group N = 45	
	n	%
Biliary atresia	10	22.2%
Budd-Chiari syndrome	9	20.0%
Autoimmune hepatitis	5	11.1%
Progressive familial intrahepatic cholestasis	5	11.1%
Congenital hepatic fibrosis	3	6.7%
Tyrosinemia	2	4.4%
Wilson disease	2	4.4%
Glycogen storage disease	1	2.2%
Sclerosing cholangitis	1	2.2%
Alagille syndrome	1	2.2%
Caroli syndrome	1	2.2%
Galactosemia	1	2.2%
Hepatitis C virus	1	2.2%
Giant cell hepatitis with AIHA	1	2.2%
Portal vein thrombosis	1	2.2%
Undiagnosed liver failure	1	2.2%

Results

Forty-five patients with ascites (64.4% males, 35.6% females, mean age 4.5 years) with different etiologies were included in the study (Table 1).

Analysis of the results showed that fever, abdominal pain and abdominal tenderness were the only significant clinical presentations in patients with SBP compared to the non-SBP group (< 0.0001). Seventeen (68%) cases of the SBP group had fever on presentation without a clinically apparent source of fever, while only 2 cases of non-SBP had fever (one case had pharyngitis and the other had otitis media). Abdominal pain and abdominal tenderness were detected in 92% and 88% respectively in the SBP group. Regarding the laboratory parameters, there was no difference regarding either complete blood count (CBC) parameters or liver function tests.

On analysis of ascitic samples, TLC and neutrophils were found to be higher in the SBP group while lymphocytes were higher in the non-SBP group. Sixty per-

cent of TLC of the SBP group were neutrophils while 97.5% of TLC of the non-SBP group were lymphocytes (Table 2). As regards culture of the ascitic sample, 20% of SBP were culture positive (5 cases had *E. coli* and 3 had *Klebsiella*) while none of the other group were positive.

On doing the GE immunoassay, GE was significantly higher in the SBP (3073.6 ±7082.78) than the non-SBP group (430 ±1029.1) (p value < 0.05). The diagnostic performance of GE in differentiating SBP and non-SBP was assessed and showed sensitivity, specificity, PPV, NPV and accuracy of 70% for each at a cut-off value of 123.5 ng/ml. Granulocyte elastase was positively correlated with TLC and neutrophil percent in the ascitic sample and showed a negative correlation with the lymphocytic percent in the ascitic sample ($p < 0.05$).

On doing LE dipstick strips, all the non-SBP group gave no coloration with LE strips while 62% of the SBP group gave coloration. 17.5% of SBP gave positive +, 22.5% gave positive ++ and 22.5% gave positive +++.

Leukocyte esterase strips in differentiating SBP and non-SBP had sensitivity of 62.5%, specificity of 100%, PPV of 100%, NPV of 72.5% and accuracy of 86.25% at cut-off value of 1 (color grade 1). Using the color grade of LE strips in predicting TLC count in ascitic sample, no coloration indicates TLC count < 750 cell/ml with accuracy of 86.75%. Color grade + indicates TLC > 750 cell/ml with accuracy 86.75%. Color grade ++ indicates TLC > 950 cell/ml with accuracy 83.35%. Color grade +++ indicates TLC > 1950 cell/ml with accuracy 75.5% (Fig. 1).

By use of the color grade of LE strips in predicting PMN cell count in ascitic sample, we found that: no coloration detects PMN count < 310 cells/ml with accuracy of 85.05%; color grade + detects PMN > 310 cells/ml with accuracy 85.05%; color grade ++ detects PMN > 691 cells/ml with accuracy 89.15%; color grade +++ detects PMN > 1577 cells/ml with accuracy 82.15% (Fig. 2).

Table 2. Ascitic fluid TLC and its differential in the SBP and non-SBP groups

	SBP $N = 40$	Non-SBP $N = 40$	p-value
TLC (cells/mm^3)	3432 ±4738	345 ±95.9	< 0.0001
Neutrophil (%)	57.33 ±29.26	27.7 ±11.51	< 0.0001
PMN cell count (cells/mm^3)	2558 ±4329	95.52 ±44.93	< 0.0001
Lymphocyte (%)	41.73 ±29.80	71.98 ±11.53	< 0.0001
TLC differential			
mainly neutrophils	24 (60.0%)	1 (2.5%)	< 0.0001
mainly lymphocytes	16 (40.0%)	39 (97.5%)	
Positive AF culture results	8 (20.0%)	0 (0.0%)	0.005

Discussion

SBP is life-threatening in decompensated liver disease, which needs close monitoring, early diagnosis and rapid intervention. Bacterial translocation is considered the key step in SBP pathogenesis; it is the "passage" of bacteria from the lumen to the mesenteric lymph nodes and thereafter to the blood stream and other extra-intestinal sites. It represents failure of a group of defensive factors to contain bacteria within the bowel. Bacterial overgrowth in association with impairment of the intestinal barrier (probably a consequence of vascular stasis due to portal hypertension), alterations of local immune defenses, slow motility of the bowel in patients with cirrhosis and reduced opsonic activity precede the episodes of bacterial translocation. More recently detection of translocation of bacterial products through the intestinal wall has been associated with production of many cytokines which cause over-activation of the sepsis syndrome pathways [4].

Fever, abdominal pain and abdominal tenderness were the only significant clinical presentations in patients with SBP compared to the non-SBP group. In agreement with our results, Bibi *et al.* (2015) found that fever was higher in SBP than non-SBP [5]. Abdominal pain and abdominal tenderness were detected in 92% and 88% respectively in the SBP group. These results were higher than those reported by Wallersted *et al.* (2007), who stated that abdominal pain and tenderness were detected in 70% of SBP cases [6]. Regarding the laboratory parameters, there was no difference regarding either CBC parameters or liver function tests.

TLC and neutrophils were higher in AF of the SBP group, while lymphocytes were higher in the non-SBP group. Sixty percent of TLC of the SBP group were neutrophils while 97.5% of TLC of the non-SBP group were lymphocytes. Abulseoud *et al.* (2016) stated that, although ascitic total WBC count increases in SBP cases, it suffers from low specificity because a large proportion of patients with sterile ascites have increased white blood cell counts [7].

GE was significantly higher in the SBP (3073.6 ±7082.78) than the non-SBP group (430 ±1029.1). GE at cut-off value of 123.5 ng/ml had accuracy of 70% in differentiating SBP from non-SBP samples. Yamazaki *et al.* (2011) found that the ROC curve showed that ascitic GE by immunoassay enabled discrimination between SBP and non-SBP, and a cut-off value of 49.5 ng/ml had a sensitivity of 85.7% and specificity of 97.7% [8]. GE was positively correlated with TLC

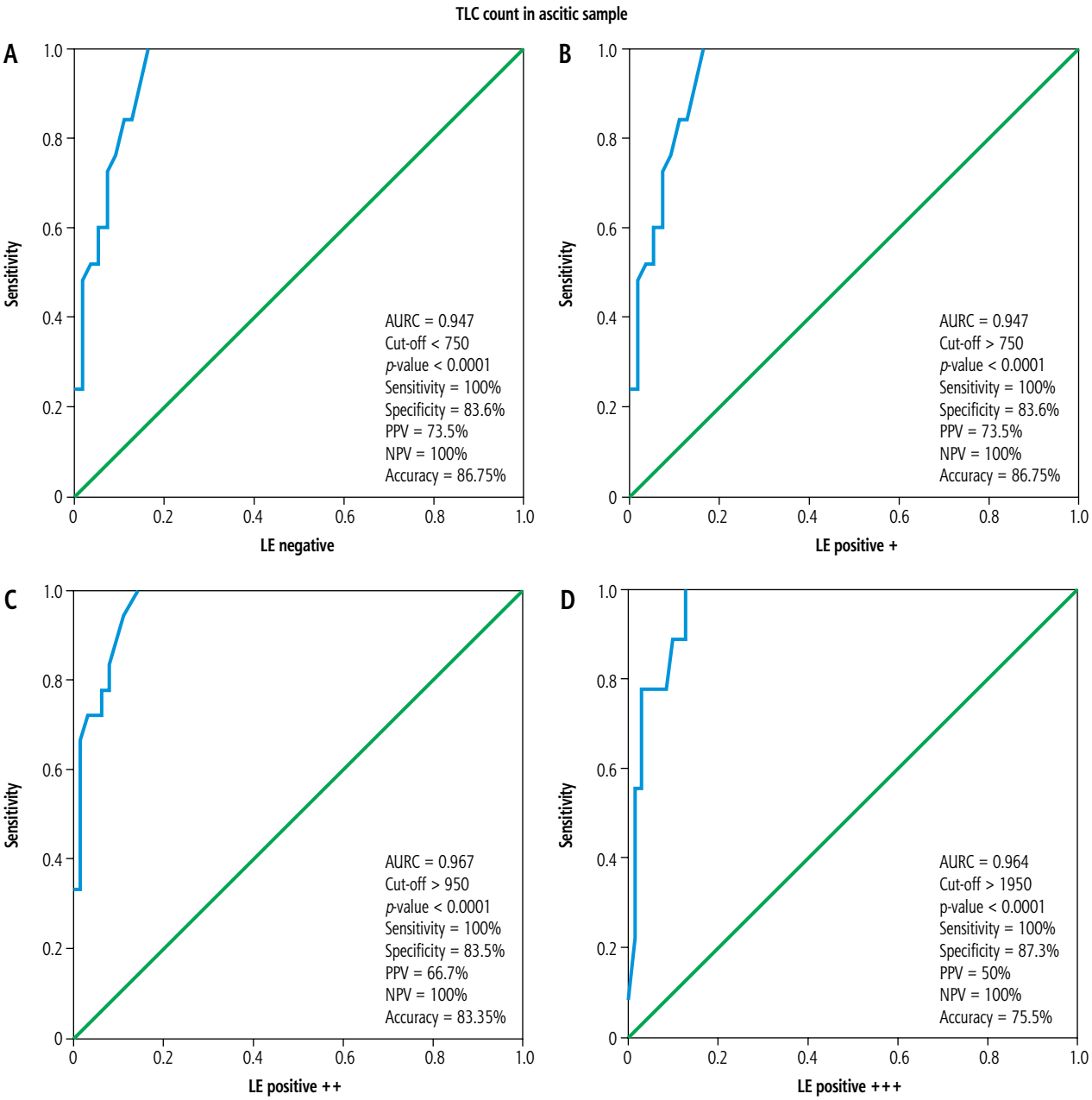

Fig. 1. ROC curve of color grades of LE strips in relation to TLC count in ascitic sample

and neutrophil percent and negatively correlated with the lymphocytic percent in AF ($p < 0.05$).

On doing LE dipstick strips, all non-SBP group samples showed no change in strip color while 62% of the SBP group showed different grades of discoloration, which led us to assess the predictive power of each color grade in predicting different levels of TLC and PMN cells at bedside.

LE strips at cut-off value of 1 (color grade 1) had an accuracy of 86.25% in differentiating SBP and non-SBP patients. Comparable results were reported by Koulaouzidis (2011), when using the same strips to diagnose SBP in 136 specimens by using grade 2 as a cut-off scale, and found the sensitivity, specificity, PPV and NPV of 83%, 99%, 91% and 98% respectively [9]. Also, Castellote and Xiol (2008) studied 228 samples of AF and diagnosed 52 episodes of SBP, using different type of strips (Autionsticksâ, A. Menarini Diagnostics, Firenze, Italy). The sensitivity and specificity of the reagent strip were 96% and 89%, respectively [10].

Conclusions

Application of leukocyte esterase reagent strips is a bedside, rapid, easy-to-use, and inexpensive method for diagnosis of SBP. It has an accuracy of 86.25% in

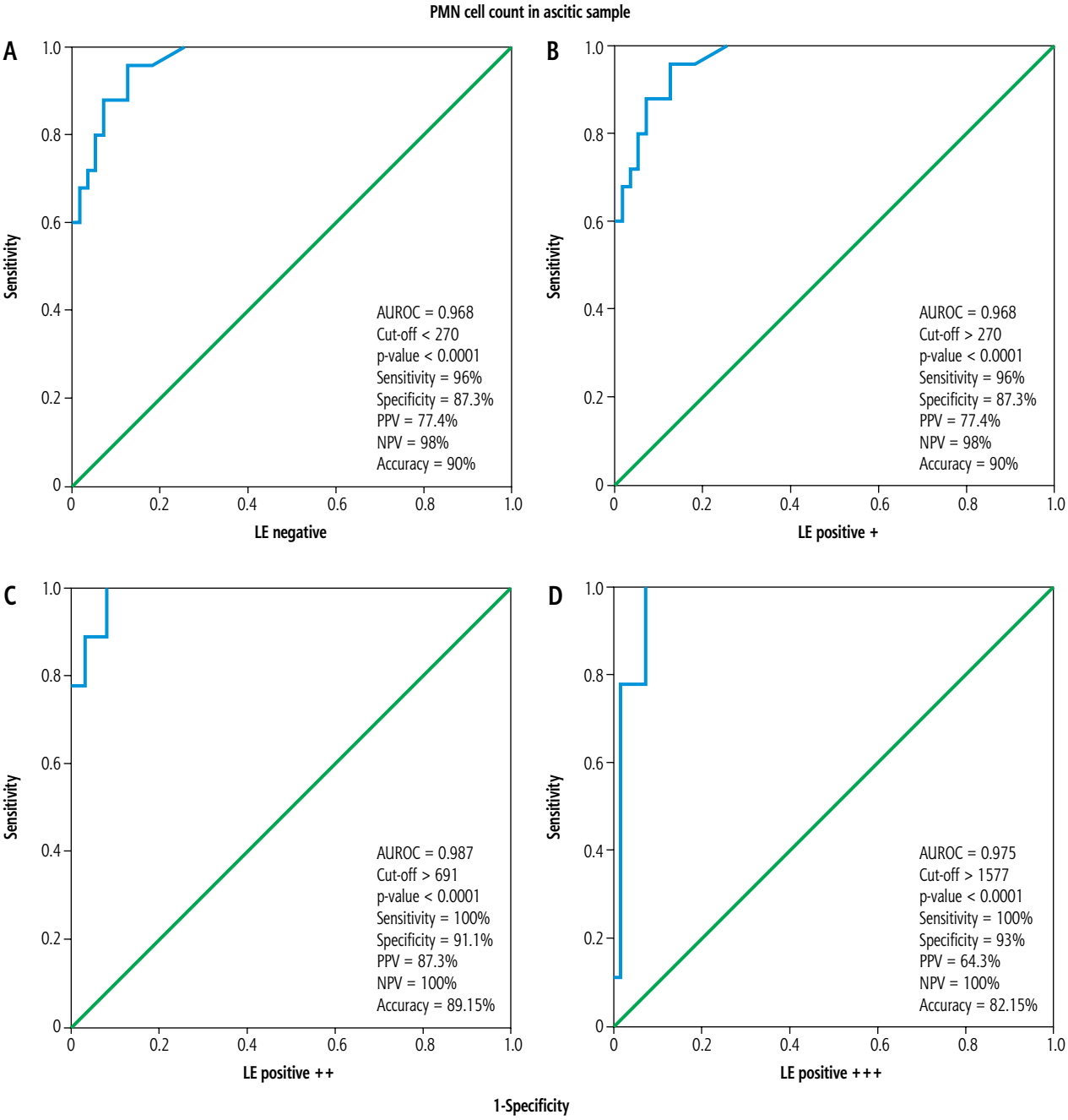

Fig. 2. ROC curve color grades of LE strips in relation to PMN cell count in ascitic sample

differentiating SBP and non-SBP, which is higher than more complicated methods such as GE latex immunoassay, which has an accuracy of 70%.

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Disclosure

Authors report no conflict of interest.

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